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Bands & Condensed Chromatin as Sites of Transcription in Polytene Chromosomes of *Drosophila*

S. C. LAKHOTIA
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005

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The question of involvement of DNA of bands and other condensed chromatin masses in transcription in polytene chromosomes of salivary glands of *Drosophila* larvae has been analysed by electron microscopy and autoradiography (at light and electron microscope level) following a 5 min *in vitro* pulse labelling with ^{3}H-uridine. Ultrastructural studies show that many of the thick bands have regions of lesser electron density within their 3-dimensional structure. Typical perichromatin granules are seen to be specifically associated with the less electron dense regions (perichromatin) of several thick and thin bands and also with the perichromatin around the condensed masses in beta heterochromatin of chromocentre. Autoradiographic studies show that certain bands and the condensed masses of beta heterochrometin incorporate ^{3}H-uridine to a significant level. Majority of the interbands are *not* labelled with ^{3}H-uridine. It is suggested that in polytene chromosomes, bands and other condensed chromatin masses (like the beta heterochromatin) may become transcriptionally active without apparent decondensation. Most of the interbands probably never transcribe.

THE distinct band-interband organization of polytene chromosomes has evoked interest in their genetic correlates. The one to one relationship of the number of bands (and interbands) with the number of genetically determined complementation groups in a given chromosome segment has been implied to suggest that a polytene chromosome band with its associated interband constitutes a unit of function[1]. One question which has repeatedly been discussed in this context is the localization of the informational part of a genetic unit in the band or the interband. Various considerations have led to the belief that the coding sequences of a genetic unit are located within the band DNA[2-6]. However, on the basis of the amount of DNA present in bands and interbands, respectively, and on other considerations, Crick[7] suggested that while the coding sequences are located within interbands, the band DNA has a regulatory role. Similarly, on the basis of light microscope (LM) autoradiographic data on ^{3}H-uridine incorporation in polytene chromosomes it has been suggested[8] that in addition to puffs nearly all interbands synthesize RNA while the bands do not. It has also been suggested that in polytene nuclei, RNA polymerase II is present in all interbands and puffs but not on bands[9-11]. On the basis of ultrastructural studies, Skaer[12] has also concluded that the transcriptionally active regions are within the interbands and puffs. Inspite of these apparently conclusive evidences for occurrence of transcription only on puff and interband regions of polytene chromosomes, a few recent observations have questioned these generalisations and have raised the possibility of RNA synthesis at certain condensed regions of polytene chromosomes. Lakhotia and Jacob[13] showed a high rate of RNA synthesis in the beta heterochromatin of the chromocentre of polytene nuclei of *D. melanogaster*. Studies on *in situ* hybridization of cytoplasmic or nuclear RNA to polytene chromosomes have shown a specific hybridization of RNA to certain bands[14]. More recently, Ananiev and Barsky[15] have shown a high degree of ^{3}H-uridine incorporation over a significant number of condensed bands of *D. melanogaster* polytene chromosomes. The present paper provides additional evidence for RNA synthesis by condensed chromatin regions in polytene nuclei of *Drosophila*. For this purpose, ultrastructural and autoradiographic (light as well as electron microscopic) studies have been done.

Materials and Methods

Salivary glands from late 3rd instar larvae of wild type strains of *D. melanogaster* and *D. nasuta* were used for these studies. For ultrastructural observations, salivary glands from *D. melanogaster* larvae were fixed with glutaraldehyde and osmium tetroxide and embedded in Epon-Araldite mixture as described earlier[16]. Ultrathin (~100 nm) sections of glands, double stained with uranyl acetate and lead citrate, were examined under transmission electron microscope. For localizing the sites of RNA synthesis, light (LM) and electron microscope (EM) autoradiographic studies have been done. For LM autoradiographic observations, salivary glands from late third instar larvae of *D. nasuta* were pulse labelled *in vitro* with ^{3}H-uridine (1mCi/ml; sp. act. 12.6Ci/mM, BARC, Trombay) for 5 min following which the glands were fixed in aceto-methanol (1 : 3) and squashed in 50% acetic acid. After removal of coverslips, the preparations were stained with carbolfuchsin and coated with Ilford LA emulsion for autoradiography. Autoradiographic exposure was between 4–7 days at 4°—6°C.

For EM autoradiography, the glands from late third instar larvae of *D. melanogaster* were pulse labelled with ^{3}H-uridine (1 mCi/ml; sp. act. 31Ci/mM,

Radiochemical Centre, Amersham) for 5 min. Following the pulse, the glands were washed, fixed and processed for EM autoradiography as described earlier[16]. Autoradiographic exposure in this case was upto 20 days.

Results

Localization of perichromatin granules — Transcription products are identifiable as ribonucleoprotein perichromatin granules (pcg) and, in general, their distribution is a good indication of active gene sites[12,17]. Pcg of various size ranges are generally distributed in puffs and a few interbands. The distribution of pcg in relation to condensed chromatin has been specifically examined in this study and the observations on this aspect are presented below and in Figs 1–3.

Figure 1 shows electron micrographs of 2 adjacent

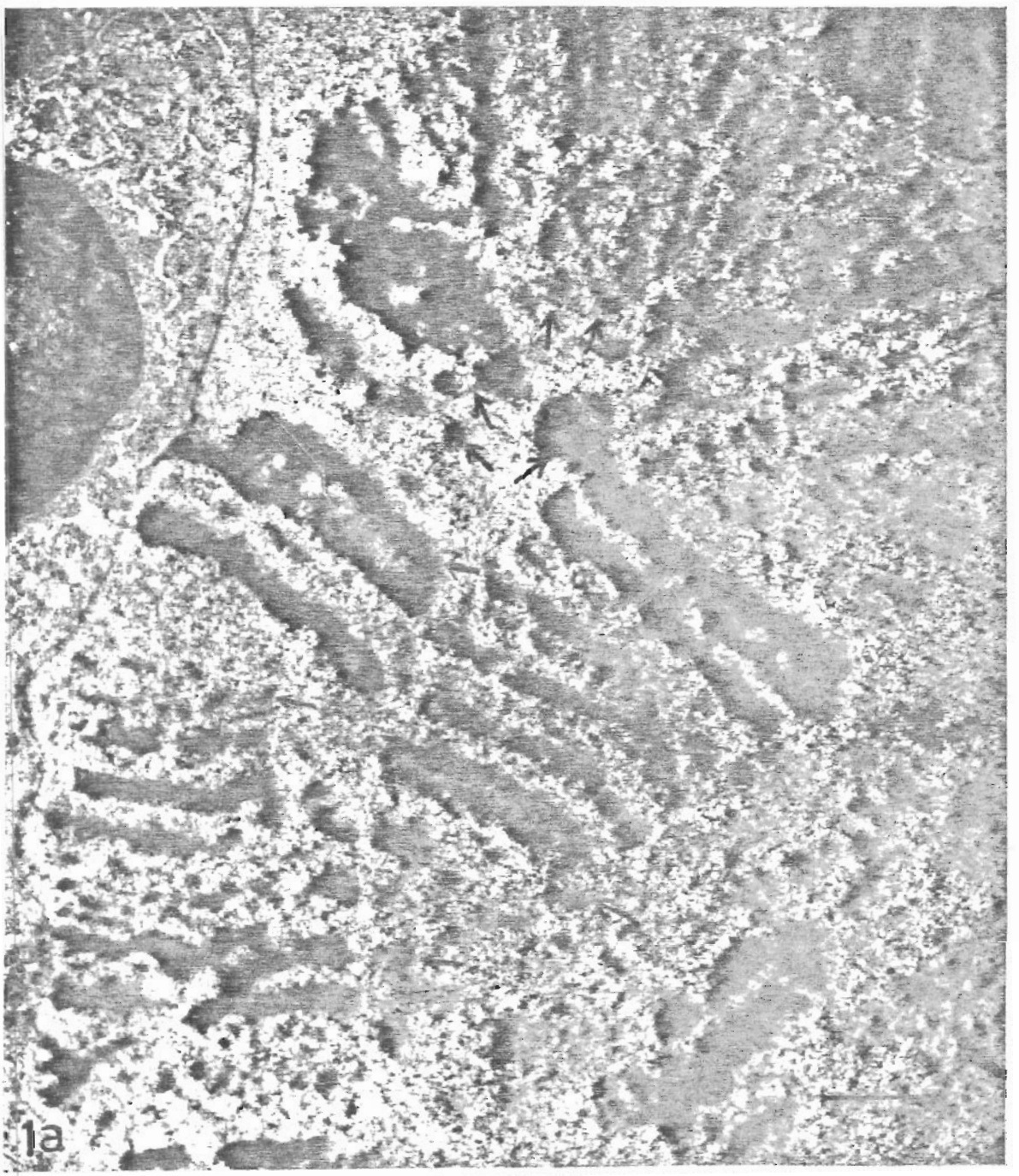
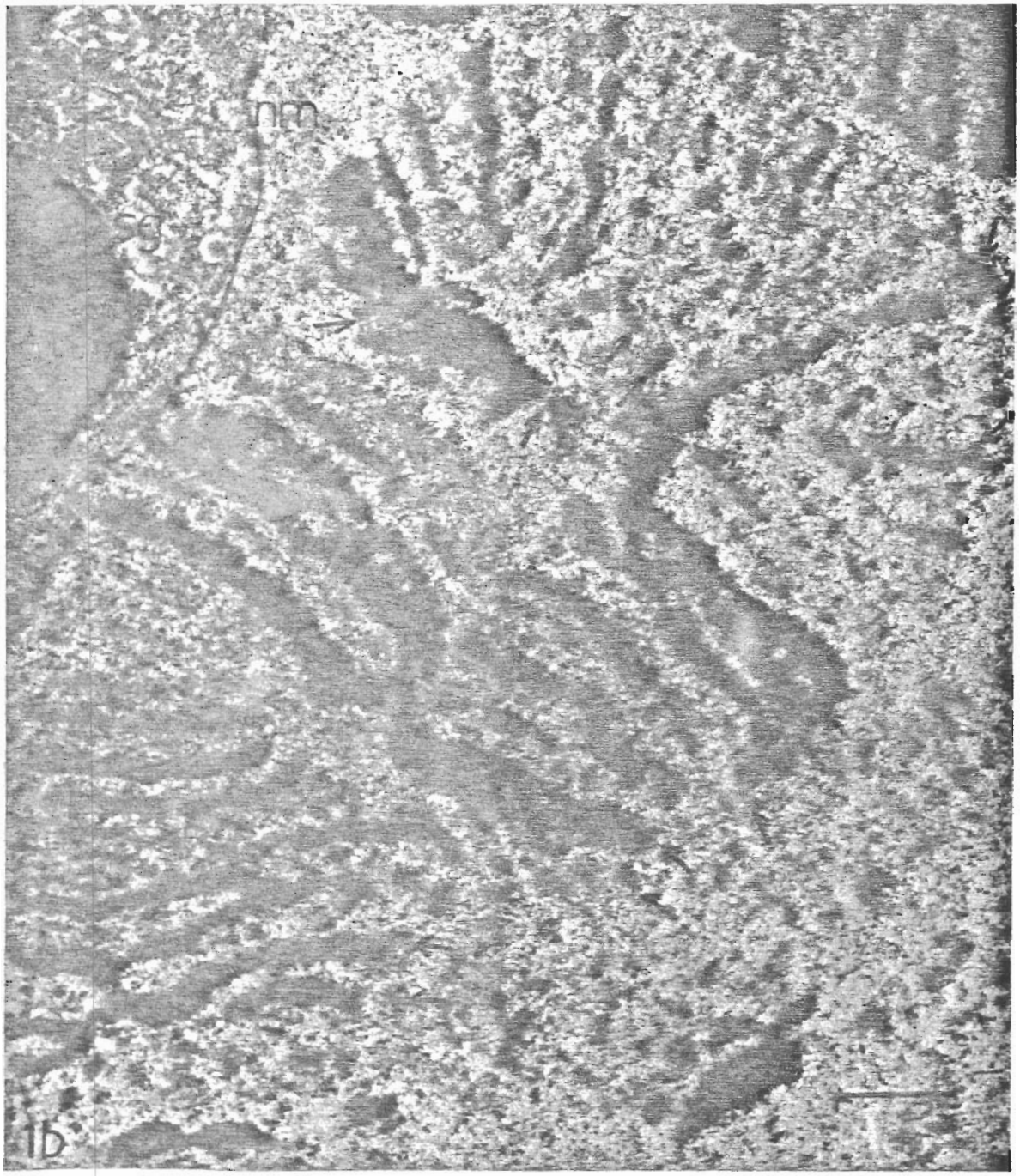


Fig. 1a-b — Electron micrographs of two adjacent sections of a chromosome segment to show that typical perichromatin granules are often associated with band regions. Many bands are dotted or enclose small regions of low electron density. While the overall banding pattern is essentially similar in the two sections the structure of certain bands varies. The bands

sections through the same unidentified chromosome segment. There is no major puff in this segment. Nevertheless, a large number of pcg is seen in many of the regions and their distribution is essentially similar in the two sections. It may be noted that a number of bands have an irregular structure and the organization of such bands is not identical in two adjacent sections—in one section, the band may appear as a homogenously condensed mass of fibrils while the same band in another section could have lateral disruptions or may contain regions of low electron density within its thickness. It is significant that specifically associated with most such bands are a large number of typical pcg. As seen in Fig. 1, the interbands adjacent to bands marked with arrows are free of pcg while in close proximity to these bands are clusters of pcg. These granules are particularly abundant in regions where the bands appear laterally broken or in areas which appear as vacuolations within the band structure.



or band regions specifically associated with perichromatin granules are indicated by arrows. Very few interband regions are seen to contain perichromatin granules. nm = nuclear membrane, sg = secretary granule. The bar represents 0.5 μm

Figure 2 shows part of two large puffs. In both the puffs, a large number of pcg (20–25 nm) is seen arranged in definite arrays. In the lower puff in Fig. 2, several small blocks of condensed chromatin are present which appear to be remnants of band/s which decondensed concomittant with puff development. The point of interest here is that these small condensed chromatin masses are surrounded by a narrow zone of perichromatin with which rows of perichromatin granules are closely associated. This suggests that these chromatin masses are involved in the formation of the associated pcg. It is noteworthy that the three interbands between the two puffs are almost free of pcg. Skaer[12] had suggested that "periochromatin granules spread along the chromosome away from their original site of synthesis and pass between the bands where they are dotted, until the granules are contained by an unbroken band above and below the puff". However, in the present study, no evidence for migration of pcg to interbands outside the puff area through the lateral discontinuities in the bordering bands could be obtained. As can be seen in Fig. 2, the two interbands (double arrows) immediately next to the two puffs are separated from the respective puff region by laterally broken bands and yet both these inter bands are nearly free of pcg. A similar distribution of pcg was seen in a few other puff regions also.

The presence of two types of pcg (20-25 nm and 40–45 nm) and RNA synthesis in the beta heterochromatin of polytene nuclei of *Drosophila* has been reported earlier[13-15]. The beta heterochromatin has an irregular organization with clumps of condensed chromatin masses and large intervening electron

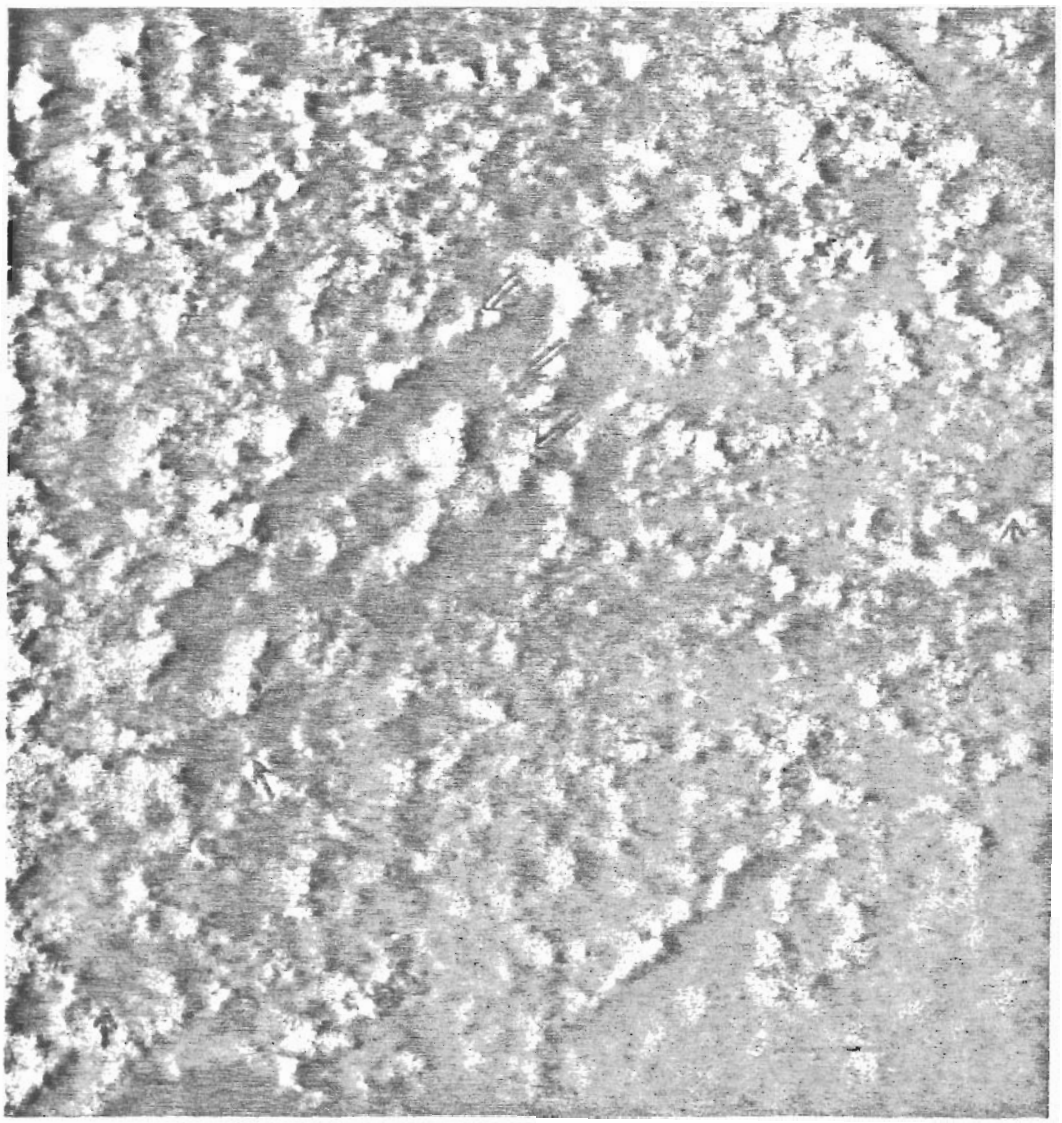


Fig. 2 — Electron micrograph of two adjacent large puffs with an abundance of perichromatin granules arranged in definite rows all over the puffs. Note the small electron dense regions (arrows) in the lower puff; rows of perichromatin granules radiate out from these chromatin bodies. Also note the three interbands (double arrows) between the two puffs; these interbands are devoid of any perichromatin granules. The bar represent 0,5 μm

lucent regions. A narrow zone of perichromatin surrounds each of the condensed chromatin masses. As can be seen in Fig. 3a, the smaller pcg (present all over the beta heterochromatin region[13]) are always clustered in rows or loops near perichromatin of the condensed masses. The large intervening spaces are totally devoid of these RNP granules. Figure 3b shows the arrangement of the larger sized pcg which are restricted to the beta heterochromatin at the base of one chromosome arm. As discussed earlier[13], these pcg are generally seen in large clusters in one or more electron lucent regions; however, in addition to such larger clusters, smaller number of these granules are also present in rows or singly, attached to the perichromatin around condensed masses in the regions (Fig. 3b). It is possible that in this region of beta heterochromatin, the larger pcg are synthesized over a wider area in close association with the perichromatin of condensed masses and subsequent to synthesis, these particles are stored or accumulated in clusters until their release to the nuclear sap.

Sites of ^{3}H-uridine incorporation — *Light microscope autoradiography*—LM autoradiographs of squash preparations of *D. nasuta* polytene chromosomes following a 5 min pulse of ^{3}H-uridine have been studied. In the present study, the right arm of the chromosome 2 (2R) has been examined in some detail. In order to obtain a greater degree of resolution of labelled sites, some preparations were made with more than the usual degree of chromosome stretching. In such stretched chromosomes, the interband regions get extended so that the bands appear more widely spaced[15,19]. Two sets of LM autoradiographs of 2R of *D. nasuta* are shown in Fig. 4 : in one set (a, c and e) one complete 2R is shown in moderately stretched condition and in the other set (b, d and f), some parts of 2R are shown in a relatively more stretched condition. As expected, the puff sites usually incorporate a much higher level of ^{3}H-uridine. In puffs in sections 47C, 48A (Fig. 4a, b) and 60B (Fig. 4e, f), the ^{3}H-uridine incorporation during the pulse of 5 min is limited to a narrow zone of the enlarged puff and most of the label in such puffs appear to be directly overlying the small darkly stained chromatin blocks. Very few interbands appear to be labelled distinctly with ^{3}H-uridine. As seen in Fig. 4, only about 12 interband regions can be considered to be labelled with ^{3}H-uridine along the entire length of 2R of *D. nasuta*. However, it may also be noted that in some of the labelled interband regions (e.g., the interbands preceding bands 50C1, 50C2, 52B5 in Fig. 4d) the silver grains appear

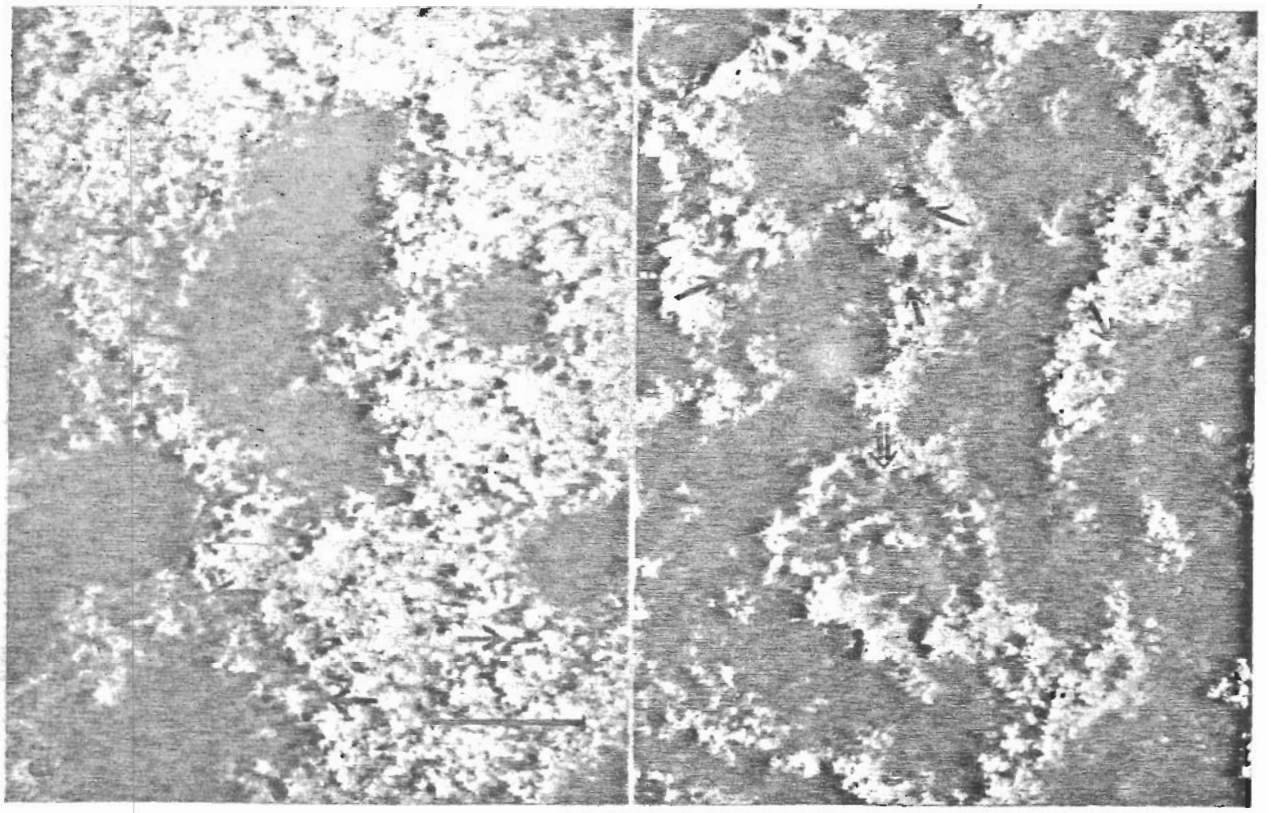
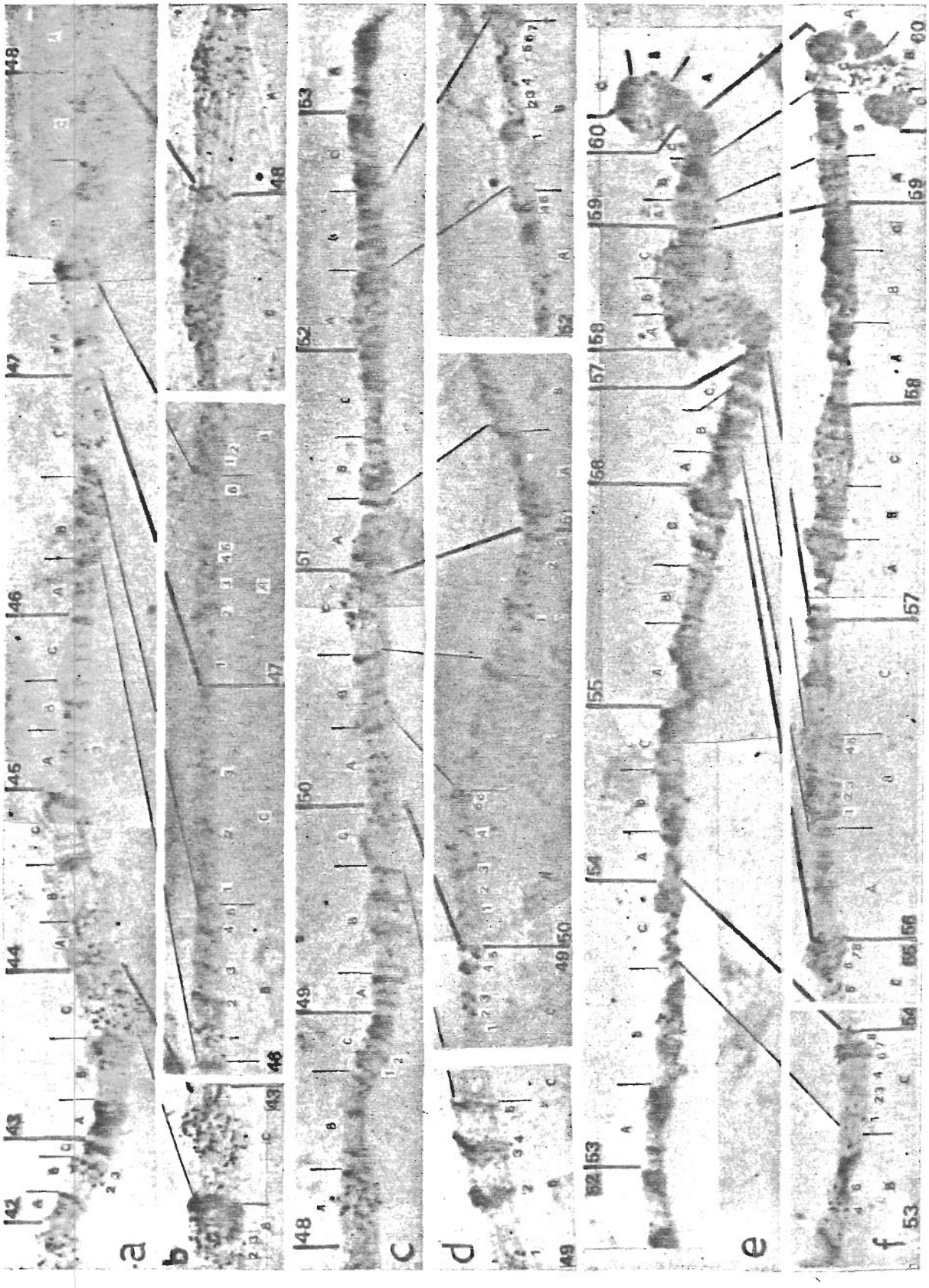


Fig. 3 a-b — Electron micrographs of two different regions of beta heterochromatin showing the relationship of perichromatin granules with the condensed chromatin masses. (*a*) The clumps of condensed masses are seen to be associated with a very large number of perichromatin granules, which at many places are arranged in rows or loops (arrows). The intervening electron lucent regions are free of these granules. (*b*) A segment of beta heterochromatin at the base of one particular chromosome is seen : one cluster of larger perichromatin granules is present in the electron lucent region (double arrow). In addition, smaller groups of similar granules are also widely distributed and are always placed very close to the electron dense masses (arrow) rather than in the intervening spaces. In both *a* and *b*, a narrow zone of perichromatin (pc) may be seen around all the condensed masses. The bar represents 0.5 μm


a
b
c
d
e
f

in a definite row and at the moment, the possibility of the presence of a thin band under the label can not be ruled out. Furthermore, some regions which appear as long interbands in stretched chromosomes, are seen as small puffs in normally spread chromosomes from the same or different larva (compare section 46C in Fig. 4a and b and section 53B in Fig. 4e and f). In no case, a major part of any interband was seen to be labelled with ^{3}H-uridine; the silver grains over the few interbands which were considered to be labelled, were always restricted to a narrow zone close to the band.

A considerable number of bands on chromosome 2R (and also on other chromosome arms) of *D. nasuta* have been seen to incorporate ^{3}H-uridine. Some of the well labelled thick bands seen in examples in Fig. 4 are : 42B2-3, 47B2, 48C2, 49C4–5, 50C1, 52A4–5, 53C6–7, 55C7–8 and 56B4–5. Examples of labelled thin bands are : 43B2–3, 45A3, 48C1, 49B1, 49C2, 50A4, 55C5, etc. Examination of stretched chromosomes (Fig. 4b, d and f) establishes beyond doubt that the labelling of bands in these cases is not due to scatter from radioactivity in the adjacent interband regions (e.g. compare the labelling in section 46B in Fig. 4a and 4b). In a few regions of 2R, the silver grains are either on the band as well as on a narrow adjacent region of interband (e.g., 49C4–5, 50C1) or only at the interface of band and interband (e.g., 52A4–5). In either cases, a major part of the interband, however, remains unlabelled.

Electron micrasocpe autoradiography — More than 50 EM autoradiographs of unsquashed salivary glands of *D. melanogaster* have been examined to ascertain the sites of ^{3}H-uridine incorporation in polytene nuclei. This analysis confirms the results obtained from LM autoradiographs described above. In EM autoradiographs of unsquashed glands, it is not generally possible to identify a given chromosome segment, and, therefore, the present observations are general without reference to a particular puff or band etc.

The nucleolus is extensively labelled and very often the silver grains over the nucleolus are arranged in definite rows (Fig. 5a), reflecting some order of the arrangement of intranucleolar DNA. The interband regions remain unlabelled in most cases (Fig. 5a,b), although a few are low labelled. In each of the autoradiogram, several bands are always labelled. Figures 5a–b, show two autoradiographs of some unidentified chromosome segments. The labelling of a number of bands in these segments is very significant. In fact, majority of silver grains in these and other EM autoradiographs are directly overlying the condensed chromatin of bands. Among the less electron dense regions, only the puffed regions are labelled to any significant level. As in the LM autoradiographs of *D. nasuta* chromosomes, in the EM autoradiographs of *D. melanogaster* chromosomes also, the silver grains in some cases are at the interface of band and interband, rather than within the limits of an interband.

The beta heterochromatin in the chromocentre region has been shown to be very active in ^{3}H-uridine incorporation[13] and as discussed above, the pcg in this region appear to be specifically associated with the condensed chromatin masses. In parallel to this, the EM autoradiographs show that the silver grains in ^{3}H-uridine labelled preparations, are specifically *on* the condensed chromatin masses (Fig. 5c); there is almost no labelling of the intervening electron lucent regions of beta heterochromatin.

Discussion

In several recent studies, it has been suggested that the transcriptionally active sites in polytene nuclei of *Drosophila* are the puffs and most or all interbands[7–12]. A common conclusion of all these studies has been that for transcription of a genetic unit localized in a band region, the band must first decondense to become a puff or an interband[10]. In the present study, however, both ultrastructural and autoradiographic evidences are obtained for a significant transcriptional activity of condensed regions (certain bands and chromocentre beta heterochromatin); Ananiev and Barsky[15] have also observed a significant level of ^{3}H-uridine uptake on band regions in *D. melanogaster*. Thus, a complete decondensation may not be a prerequisite for transcription to occur on a condensed region, like bands or the chromatin masses of beta heterochromatin.

Examination of serial sections of unsquashed chromosomes reveals that the organization of chromatin fibrils constituting a band may not be homogenous over the entire 3-dimensional structure of a band. Normally, in a band region the internal cohesion of different lateral strands making up a polytene chromosome is believed to give a definite morphology to a given band, either as a continuous line of even thickness or as a row of equidistant dots[2]. The presence of irregular electron lucent areas at various places within the structure of a given band would suggest that in these bands, some segments of the lateral components ae not as condensed as others. The presence of trypical pcg in the vacuolated or clear areas within or around some dark bands is significant in this context. In some cases, Skaer[12] also observed groups of pcg opposite breaks in bands, but suggested this to be due to passage of pcg from one active interband to another through breaks in a condensed band. It was considered[12] unlikely "that the band has decondensed at the gap containing perichromatin granules, and that these granules have spread into the neighbouring interband". In the present study, no evidence for a regular spreading of pcg from their site of synthesis to other chromosomal regions has been found. It seems likely that with the exception of a few sites, the distribution of pcg reflects

Fig. 4 — LM autoradiographs of chromosome arm 2R of *D. nasuta* to show sites of ^{3}H-uridine incorporation. On moderately stretched complete 2R is shown in 3 parts (a, c, and e) while smaller segments of 2R in more stretched chromosomes are shown in b, d and f. The numbering of different regions is on the basis of our detailed photomap (unpublished) of *D. nasuta* polytene chromosomes. The 2R is divided into 20 divisions (from chromocentre to free tip, divisions 41 to 60); each division is further subdivided into 3 sections (A, B, C); each section starts with an interband and ends with a band. The individual bands are serially numbered in some regions to facilitate identification of the bands or interbands which are labelled (see text), x 1600

their original sites of synthesis. Unlike Skaer's[112] contention, it is suggested that there may indeed be small regions of local decondensation within bands and RNA synthesis proceeds in such band regions. This view is supported by several observations. In some cases, the bands containing or associated with pcg are bordered on either side by interbands which are almost devoid of pcg—this makes it unlikely that the pcg in these bands are the result of transcriptional activity of adjoining interbands. The localization of pcg in beta heterochromatin also supports the view that transcription products can arise in close association with condensed chromatin.

The brief duration of ^{3}H-uridine pulse (5 min) and the increased resolution offered by autoradiography of stretched chromosmes and by EM autoradiography ensure that the location of silver grains in the autoradiograms represent the original sites of transcrip-

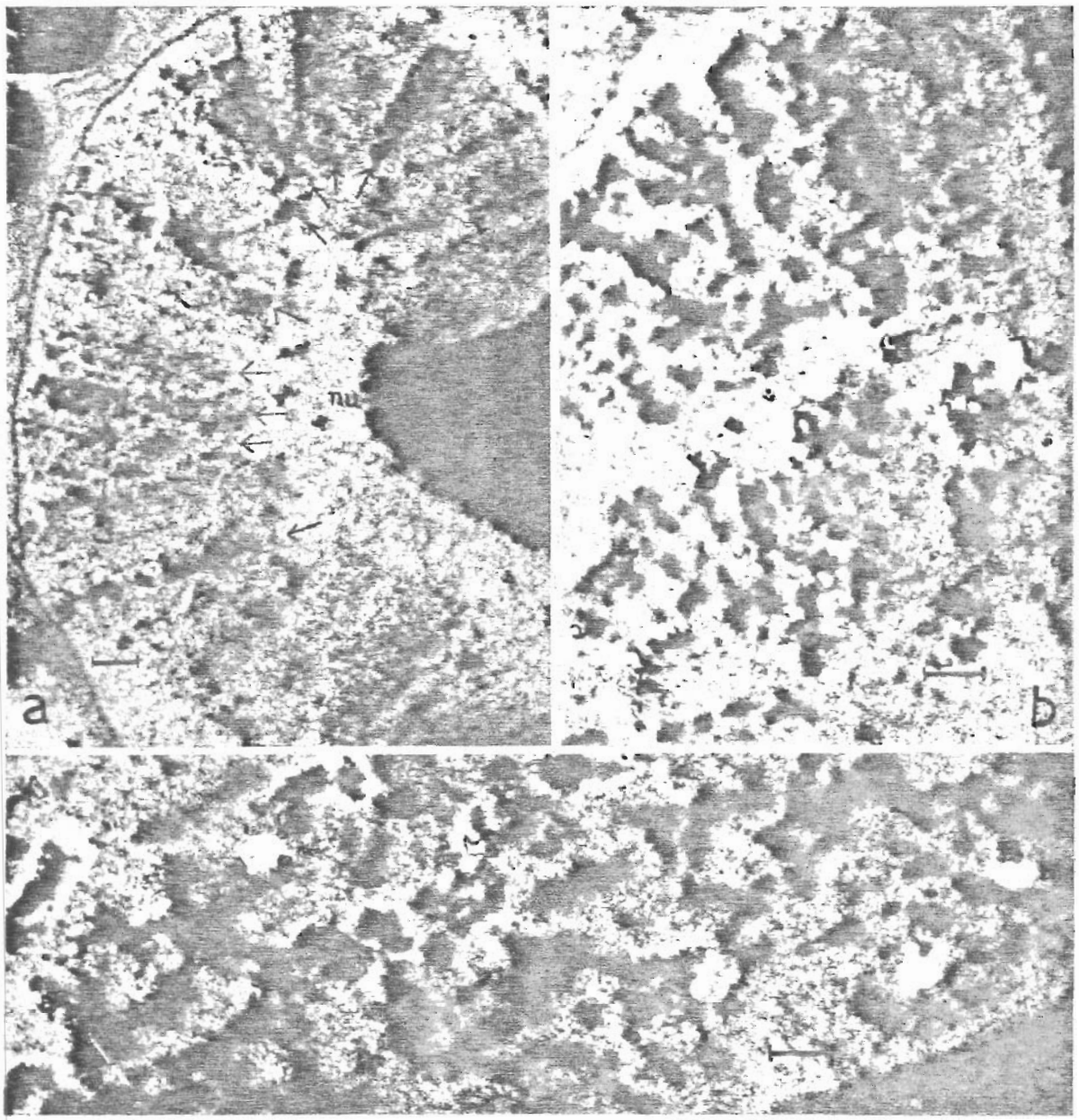


Fig. 5—^{3}H-uridine labelled EM autoradiographs of salivary gland nuclei of *D. melanogaster* to show labelling of condensed regions. (*a*) A part of nucleolus (nu) and a chromosome segment are seen; the nucleolus is heavily labelled while the silver grains over the chromosome segment are seen directly over many bands (arrows). Almost none of the interbands are labelled. (*b*) Parts of two chromosome segments are seen— in both, nearly all the silver grains are over the condensed chromatin of bands. (*c*) A part of the beta heterochromatin is seen. The silver grains are located on the condensed chromatin masses of beta heterochromatin (closer to the perichromatin zone) rather than on the electron lucent regions. The bars represent 1 μm

tional activity. The unambiguous labelling of condensed chromatin of certain bands and the beta heterochromatin masses seen by LM as well as EM autoradiography and the similarity between the distribution of pcg and ^{3}H-uridine labelling sites, clearly indicate that the electron dense regions can transcribe without complete decondensation. Furthermore, Zhimulev and Belyaeva's[8] conclusion that all interbands are transcriptionally active, does not find support in present study since very few interband regions were seen to incorporate ^{3}H-uridine. On the basis of a detailed autoradiographic study of stretched chromosomes, Ananiev and Barsky[15] have also concluded that in the tip region of polytene X-chromosome of *D. melanogaster*, no independently transcribing interband region exists.

Jamrich *et al.*[10,11], have examined the localization of RNA polymerase II in polytene chromosomes by immunofluorescence technique and they have concluded that this enzyme activity is localized only in puffs and interbands. However, from the Figs. 2 and 4 of Jamrich *et al.*[10] it is obvious that while most of the very thick bands lack any resolvable RNA polymerase II activity, many of the smaller and less prominent bands show as intense fluorescence as the interbands and puffs. Besides, it is difficult to resolve if the peripheral parts of some thick bands contain RNA polymerase activity as suggested by present observations. It is also significant that in the initial study[9], a number of bands were observed to fluorescence brightly with antibodies for RNA polymerase II. However this band fluorescence was later[10] interpreted as a fixation artefact. Furthermore, Jamrich *et al.*[11] found the chromocentre region to be poor in RNA polymerase II activity and have ascribed this to transcriptional inactivity of chromocentre heterochromatin. However, a major part of the chromocentre in polytene nuclei of *Drosophila* is known to be active in RNA synthesis[13,18,20,21]. It appears, therefore, that a lack of fluorescent staining with antibodies for RNA polymerase II may not necessarily reflect transcriptional inactivity of a region like the beta heterochromatin and also, a localization of RNA polymerase II at a site does not necessarily mean that these sites *are* synthesising RNA. Thus, although RNA polymerase II is reported[10,11] to be present on all interbands, very few of them have been seen to be actually synthesizing RNA.

At this stage it will be relevant to consider some aspects of polytene chromosome organization. The bulk of cytogenetic data[2-6] supports the view that the informational sequences of genetic units are located in band DNA. This view also finds support from results of studies[14,20,21] on *in situ* hybridization of nuclear and/or cytoplasmic RNA with polytene chromosome since in most cases the hybridization occurs specifically at band regions rather than the interband regions. However, views have also been expressed that most or all interbands are transcriptionally active and contain the informational sequences[7,8,22]. Zhimulev and Belyaeva[23] have further suggested that the bands are not structural and functional units of chromosomes, rather they represent chromosome regions which are inactive at a certain point of time (also see ref. 24). An essentially similar model of polytene chromosome organization has been suggested by Jamrich *et al.*[10] However, the constancy of banding pattern in polytene chromosomes appears to be fairly well documented[2]. Furthermore, the present data and those of Ananiev and Barsky[15] show that almost none of the interbands may be active in transcription to any significant level, while certain unpuffed band regions do transcribe. It is, therefore, suggested that the interbands represent permanent structural features of polytene chromosome organization and they may contain sites with which RNA polymerase remains associated without necessarily transcribing. This view would explain the immunofluorescent localization of RNA polymerase II[10] on almost all interbands. The informational DNA of a given cytogenetic unit is visualized to be located mainly in bands, presumably close to the junction of band and interband[25]. Depending upon the regulatory events, a part or all of the multiple strands of informational DNA in a given polytene band becomes transcriptionally active. If just a few of the strands are activated, there is only a partial decondensation of a band and these decondensed strands may give rise to "vacuolations" or lateral disruptions within a band structure, as seen in the electron micrographs of presumably active bands. Such partially decondensed bands will also show ^{3}H-uridine uptake. If most or all of the 2^n strands of a polytene band are activated, a small or big puff may result, depending upon the size of the transcription unit, the rate of synthesis and turnover of the transcription product.

Acknowledgement

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